

AD _____

Award Number: DAMD17-01-1-0433

TITLE: Molecular Tracking of Proteolysis During Breast Cancer
Cell Extravasation: Blockage of Therapeutic Inhibitors

PRINCIPAL INVESTIGATOR: Rama Khokha, Ph.D.

CONTRACTING ORGANIZATION: University Health Network
Toronto, Ontario M5G 2C4 Canada

REPORT DATE: August 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030313 186

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE

August 2002

3. REPORT TYPE AND DATES COVERED

Annual (1 Aug 01 - 31 Jul 02)

4. TITLE AND SUBTITLE

Molecular Tracking of Proteolysis During Breast Cancer Cell Extravasation: Blockage of Therapeutic Inhibitors

5. FUNDING NUMBERS

DAMD17-01-1-0433

6. AUTHOR(S)

Rama Khokha, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University Health Network
Toronto, Ontario M5G 2C4 Canada

E-Mail: rkhokha@uhnres.utoronto.ca

8. PERFORMING ORGANIZATION
REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING
AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)

Elucidating the proteolytic effectors responsible for focal proteolysis at the cellular/pericellular matrix interface during metastasis is an on-going challenge. We have begun to dissect the molecular dynamics of proteolysis required for tumor cell transendothelial migration. MDA-MB-231 (metastatic) cells are far more efficient at TEM compared to MCF-7 (non-metastatic) cells. MMP-2, MMP-9, MT1-MMP and TIMP-2 appear active participants during breast cancer TEM. Natural (TIMP-1, TIMP-2) as well as synthetic MMP inhibitors significantly reduced TEM of both MCF-7 and MDA-MB-231 cells. Serine inhibitors also significantly reduce TEM. Furin is detectable intracellularly as well as at the cell surface of MDA-MB-231 cells. Future experiments will focus on identification of molecules that are necessary for complex formation of the MMPs at the cell-matrix interface during TEM.

14. SUBJECT TERMS

breast cancer metastasis, matrix metalloproteinases, adhesion molecules, confocal microscopy, breast cancer

15. NUMBER OF PAGES

5

16. PRICE CODE

17. SECURITY CLASSIFICATION
OF REPORT

Unclassified

18. SECURITY CLASSIFICATION
OF THIS PAGE

Unclassified

19. SECURITY CLASSIFICATION
OF ABSTRACT

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	4
Reportable Outcomes.....	4
Conclusions.....	5

Introduction:

Elucidating the proteolytic effectors responsible for focal proteolysis at the cellular/pericellular matrix interface during metastasis is an on-going challenge. Here we dissect the molecular dynamics of proteolysis required for tumor cell transendothelial migration (TEM).

Body:

We have completed the determination of the MMP profile for MCF-7 and MDA-MB-231 breast cancer cells as well as human lung microvascular endothelial cells by Zymography. This has been performed on individual cell lines and TEM co-cultures. Verification of key MMP and TIMP expression in these cells has been further obtained at the protein level by western blotting. Both MMP-2 and MMP-9 are present, and among TIMPs, TIMP-2 is an important molecule. MT1-MMP is present only in MDA-MB-231 cells suggesting the involvement of trimolecular complex. We are currently assessing the quality of immunofluorescence staining in TEM cultures using antibodies from a number of sources (commercial and those obtained as gifts from investigators). While MMP-2, MMP-9 and MT1-MMP antibodies from Oncogene and Chemicon have worked well, an antibody from Dr. W. Garten (Marburg University, Germany) has been instrumental for detecting furin in our TEM system. We have obtained data on biological and synthetic MMP-inhibitors as well as serine inhibitor mediated reduction of breast cancer cell transmigration. MMP inhibitors, serine inhibitors, TIMP-1 and TIMP-2 show a statistically significant reduction in TEM of breast cancer cells. Surprisingly, TIMP-1 and TIMP-2 did not exert additive inhibitory effect. We also have optimized conditions for assessing MMP-based fluorogenic substrate digestion in TEM cultures by confocal microscopy.

Key research Accomplishments:

- Determination of MMP profile in isolated and TEM cultures
- Validation of identified MMPs by western blotting
- Identification of antibodies for good quality immunofluorescence signal
- Inhibition of breast cancer cell TEM by synthetic inhibitors
- Optimization for MMP-mediated substrate digestion in TEM assay

Reportable Outcomes:

We are in the process of writing our first manuscript supported by this grant

Conclusions:

Optimization of conditions for non-metastatic (MCF-7) and metastatic (MDA-MB-231) breast cancer cells in our TEM assay, at several levels as described above, forms the foundation for future experiments. We have narrowed the MMPs implicated in this process, identified the reagents to be used, and developed a protocol for quantifying substrate digestion. This is in keeping with the milestones projected.